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Specification as originally filed, with Application for Patent Serial No: **2,228,821**, on April 16, 1998, by **NORZYME INC.**, assignee of Zhibo Gan and Ronald Marquardt, for "Pin Method for Measuring Bioactive Substance".

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Pin method for measuring bioactive substance

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ABSTRACT OF THE DISCLOSURE

This present invention is for a method referred to as the pin method that greatly simplifies the detection and determination of the identity, amount and activity of molecules with biological activity. It is concerned with use of a device that will facilitate and simplify the measurement of the amount and activity of enzymes, enzyme inhibitors, lectins, receptors and other biologically active substances using a one-step technique for isolating a reactant from a product after completion of the reaction. The method involves the insertion of a pin-like device whose surface is coated with a reactant into a reaction vessel containing the appropriate compounds, allowing the reaction to proceed for a given period of time and then withdrawing the pin-like device from the reaction vessel. The amount of labeled products or reactants remaining in the reaction vessel can be directly measured without additional steps.

BACKGROUND OF THE INVENTION

It is of great importance in all field and disciplines of the life sciences to utilize the appropriate qualitative and quantitative analytical techniques for the detection, identification, and measurement of the concentration of a wide variety of biologically important molecules. These analytical techniques can be utilized in many different types of assays including those for enzymes, receptors, lectins and inhibitors, etc.

All chemical reactions in living systems are virtually catalyzed by enzymes, and the assay of enzyme activity is probably one of the most frequently encountered procedures in biochemistry. Most enzyme assays are carried out for the purpose of estimating the amount or activity of an enzyme present in a cell, tissue, other preparation, or as an essential part of an investigation involving the purification of an enzyme. The current assay methods have been developed based on the physical, chemical and immunological properties where they can be detected using photometric, radiometric, high performance liquid chromatographic, electrochemical assays, etc. (Eisenthal, R. and Danson, M. J., 1993). Although the methods basically fulfill the many essential requirements for routine analysis, there are, among those, the varying disadvantages of low sensitivity (Brenda Oppert et al, 1997), multiple steps (Twining, S. S., 1994; Pazhanisamy, S. et al, 1995) and steps that are tedious and time-consuming (Fields, R., 1976). Immunoassays have been widely using in human clinical tests and therapeutics, agriculture, food, veterinary and environmental diagnostics (Deshpandes, S. 1996). In the most cases, immunoassays are effective and valid (Cleaveland, J. S. et al 1990), but in some cases they are not suitable, for example, in the determination of enzyme activity. This occurs because the

binding assays for antibody and antigen (enzyme) can only be used to measure the concentration of an antigen (enzyme) but not its activity. It is of important to know the catalytic activity of an enzyme and not just the amount of the enzyme as a given amount of the enzyme may have a widely varying activity depending on reaction conditions. Also antibodies tend to react only with structurally similar antigens such as a specific enzyme. Therefore, it is often not possible to quantitate the amount of an enzyme from a related species using immunoassays.

Pharmaceutical industries usually utilize conventional methods mentioned above to screen compounds for discovering drugs. This process is slow due to the several steps required and the large amount of compounds needed to be tested; on a good day, a lab might test 100 to 1,000 compounds. In the race to commercialization, pharmaceutical manufacturers are facing great pressure to reduce the time to discover new clinical drugs, cut assay costs, and screen more compounds and against more targets. Therefore, there is a very high demand to develop new methods to meet the requirements of a high throughput screening (HTS). Jones et al (1997) described a method using quenched BODIPY dye-labeled casein as a substrate for determining the activities of protease, which is sensitive and amenable to automation. The degree of quenching of the fluorescent tag is crucial in this method. If there is not enough quenching due to poor conjugate or degradation of the fluorescence-labeled substrate under storage, etc. the assay will not be very useful. Also this procedure has relatively high background values which reduce its sensitivity. Another example of a potentially useful high throughput assay was made by Marquardt, et al (PCT/US97/07983). The method involves many steps of coating wells of a microplate, washing the wells, adding biologically active substance to wells, washing the wells once more, adding the indicator enzyme to wells, washing the wells again and adding a color

development reagent. The assay cannot be readily used in assays requiring rapid analysis.

A new assay method not only having potentially excellent sensitivity but being suitable for high throughput assays is preferable. This invention outlines a procedure that can achieve these goals.

SUMMARY OF THE INVENTION

This invention provides a new method for the qualitative and quantitative analysis of bioactive substances. The assay method is based on a one-step procedure for separating the reactants from the products (resultants) after completion of the reaction followed by measurement of the amount of a labeled reactant or labeled product that has been left in the reaction vessel. The device consists of two parts, one of which is a reaction vessel and other is a pin-like device that can fit into the reaction vessel. The reaction begins by insertion of the pin-like device that is coated with a reactant into the reaction vessel containing other reactants than those coated on the pin-like surface. The said pin-like surface is taken out from the reaction vessel to stop the reaction. The amount of the labeled products or labeled reactant remaining in the reaction vessel can be determined according to the intensity of its label which can be fluorescent, luminescent and chromogenic molecules or radioactive tag, etc. The amount of the label in the reaction vessel is directly or reciprocally proportional to the activity or amount of the bioactive substance that is to be measured.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for the detection, identification and

measurement of the amount or activity of biologically active molecules via a one-step separation of reactants from products using a device comprising a pin-like device whose surface is coated with a biologically active substance and a reaction vessel containing the other substances used for the reaction.

1. An assay in which the surface of a pin-like device is coated with reactant 1 which is an acceptor of a moiety from reactant 3. Reactant 3 is a substrate for reactant 2 having a biological activity. When the coated pin-like device is inserted into a reaction vessel, the labeled moiety from reactant 3 is transferred to reactant 1 due to the biological activity of reactant 2 acting on reactants 1 and 3. The amount of labeled reactant 3 remaining in the reaction vessel can be directly determined without an additional step and is reciprocally proportional to the activity of reactant 2 after the pin-like device containing the labeled moiety from reactant 3 has been taken out of the reaction vessel.

The reactions for the transfer of a moiety between reactant 1 and reactant 3 by reactant 2 is interfered with by reactant 4 which is an inhibitor of reactant 2. The amount of labeled reactant 3 remaining in the reaction vessel is directly proportional to the amount of reactant 4 (inhibitor) after the pin-like device containing the labeled moiety from the reactant 3 has been taken out of the reaction vessel.

2. An assay in which the surface of a pin-like device is coated with reactant 1 which is a labeled substrate for reactant 2 having a biological activity. Insertion of the pin-like device containing reactant 1 into a reaction vessel containing reactant 2 and other compounds essential for the reaction results in the initiation of a reaction in which labeled products of the reaction are

released into the reaction vessel. The amount of the released label in the reaction vessel can be directly measured without an additional step and is proportional to the activity of reactant 2 after the pin-like device has been taken out of the reaction vessel.

The reaction of hydrolyzation of reactant 1 coated on the surface of the pin-like device by reactant 2 is interfered with by reactant 3 which is an inhibitor of reactant 2. The amount of the released label from reactant 1 to the reaction vessel is reciprocally proportional to the amount of reactant 3 (inhibitor) after the pin-like device has been taken out of the reaction vessel.

3. An assay in which the surface of a pin-like device is coated with reactant 1 which is a binding ligand for product 1. Product 1 is formed from reactants 3 linked with either a label or a binding agent capable of binding specifically to reactant 1. Product 1 is caused by the biological activity of reactant 2 in a reaction vessel acting on reactant(s) 3. The coated pin-like device is inserted into the reaction vessel containing product 1 and other non-reacted reactants to bind the binding agent. After the pin-like device has been taken out of the reaction vessel, the amount of labeled non-reacted reactant 3 remaining in the reaction vessel can be directly measured without an additional step and is reciprocally proportional to the activity of reactant 2

The reactions of formation of product 1 from reactants 3 due to biological activity of reactant 2 is interfered with by reactant 4 which is an inhibitor of reactant 2. The amount of labeled non-reacted reactant 3 remaining in the reaction vessel is directly proportional to the amount of reactant 4 (inhibitor) after the pin-like device has been taken out of the reaction vessel.

4. An assay in which the surface of a pin-like device is coated with reactant 1 which is a binding ligand for reactant 3 being the labeled form of reactant 2. Competitive binding reactions between reactant 2 and reactant 3 for reactant 1 or between reactant 2 and reactant 1 for reactant 3 are

initiated when the pin-like device bound using reactant 1 is inserted into a reaction vessel containing a unknown amount of reactant 2 and a known amount of reactant 3. The amount of the labeled reactant 3 remaining in the reaction vessel can be directly measured without an additional step and is directly proportional to the amount of reactant 2 after the pin-like device has been taken out of the reaction vessel.

The reactions of the competitive binding between reactant 2 and reactant 3 for reactant 1 or between reactant 2 and reactant 1 for reactant 3 is interfered with by reactant 4 which is an inhibitor of reactant 2. The amount of the labeled reactant 3 remaining in the reaction vessel is directly proportional to the amount of reactant 4 (inhibitor) after the pin-like device has been taken out of the reaction vessel.

EXAMPLES

The following examples are for an illustrative purpose only, and not to limit the scope of the invention.

Example 1

The assay of protein kinase A and its inhibitor using the pin method

Materials: hydrolyzed and partially dephosphorylated casein, protein kinase 3' : 5'-cyclic AMP dependent (PKA), cyclic AMP, protein kinase inhibitor, Na_2HPO_4, NaH_2PO_4, NaCl, Tween-20 are from Sigma. ^{32}P-ATP is from New England Nuclear. The 96-well microplate and 96-pin lid are from VWR Canlab.

Method:

1. Preparation of a coated 96-pin lid:

(1). 5 g skim milk powder dissolved in 100 ml phosphate buffer saline (PBS, pH 7.2) is added to the wells of a microplate and incubated at 37^0 C for 2 hr. The microplate is washed three times with PBST (PBS + Tween).

(2). Casein is dissolved in PBS to the concentration 5 ug/ml and 100 ul/well is added to each well of the microplate blocked with skim milk. The 96-pin lid is inserted into the wells of the microplate and incubated at 37^0 C for 3 hr, and is then taken out and rinsed with PBST.

2. Protein kinase assay:

(1). A series of concentrations of protein kinase in phosphate buffer (PB pH 7.2, cAMP, ^{32}P-ATP) are added to the wells of a microplate (100 ul/well).

(2). The reaction is initiated by inserting the 96-pin lid coated with casein. During incubation at 37^0 C for 30 min, ^{32}P is transferred from ^{32}P-ATP to casein by protein kinase. The 96-pin lid is then taken out of the wells of the microplate to stop the reaction.

(3). The radioactivity of ^{32}P-ATP remaining in the wells of the microplate is counted in a scintillation counter and is reciprocally proportional to the activity of the protein kinase.

3. Protein kinase inhibitor assay:

(1). Varying concentrations of an inhibitor in the phosphate buffer (PB pH 7.2, cAMP, ^{32}P-ATP) are added to the wells (50 ul/well) of a microplate. Negative and positive control are also included.

(2). A fixed amount of protein kinase A (50 ul/well) is added to each well containing inhibitor and the controls. Inhibition of protein kinase activity by the inhibitor will occur.

(3). The reaction of phosphate transferring is initiated by inserting the 96-pin lid coated with casein followed by incubation at 37^0 C for 30 min. Protein kinase catalyzes the transfer of ^{32}P from ^{32}P-ATP to casein with the degree of transfer being reciprocally related to the concentration of the inhibitor. The 96-pin lid is taken out of wells of the microplate to end the reaction.

(4). The radioactivity of ^{32}P-ATP remaining in the wells of the microplate is counted in a scintillation counter and is directly proportional to the amount of the inhibitor.

This assay is a specific example of the general assay given in section 1 of the "Detailed Description of the Invention".

Example 2

Fluorescent assay of protease and protease inhibitors using the pin method

Materials: proteinase K, elastase, protease XIII, papain, trypsin, pepsin, casein, dimethyl sulfoxide (DMSO), Na_2HPO_4, NaH_2PO_4, NaCl, Tween-20, citrate, ovomucoid, aprotinin are from Sigma. NHS-coumarin is from Molecular Probes. The 96-well microplate and 96-pin lid are from VWR Canlab.

Method:

1. Preparation of a fluo-casein: 5 mg NHS-coumarin in 100 ul DMSO is mixed with 10 mg casein in PBS (pH 7.2) in a microcentrifuge tube and incubate at room temperature for 3 hr.

2. Preparation of a coated 96-pin lid:

(1). 5 g skim milk powder dissolved in 100 ml PBS (pH 7.2) is added to the wells of a microplate and incubated at 37^0 C for 2 hr. The microplate is washed three times with PBST.

(2). Fluo-casein is dissolved in PBS to the concentration 5 ug/ml and 100 ul/well is added

to each well of the microplate blocked with skim milk. The 96-pin lid is inserted into the wells of the microplate, incubated at 37° C for 3 hr, then taken out and rinsed with PBST.

3. Protease activity assay:

(1). A series of concentrations of a protease in a buffer (100 ul/well) are added to the wells of a microplate.

(2). The reactions are initiated by inserting the 96-pin lid coated with the fluo-casein and incubated at 37° C or room temperature for 30 min. The fluo-casein is hydrolyzed by protease to release the fluorescent labels into the reaction vessel. The 96-pin lid is taken out of the reaction vessel to stop the reaction.

(3). The fluorescent intensity of the label in the wells of the microplate is measured with a fluorometer and is directly proportional to the activity of the protease.

4. Protease inhibitor assay:

(1). Varying amounts of the protease inhibitor in the buffer (50 ul/well) are added to the wells of a microplate. Negative and positive controls are included.

(2). A fixed concentration of the protease in the buffer (50 ul/well) is added to the wells containing inhibitor and the controls. The inhibition of the protease by the inhibitor reduces the amount of label released into the reaction mixture.

(3). Hydrolysis of the casein by the protease is initiated by inserting the 96-pin lid coated with fluo-casein. The sample is incubated at 37° C or room temperature for 1 hr. The fluo-casein is cleaved by the residual activity of the protease after inhibition to release the fluorescent label into the reaction vessels. The 96-pin lid is taken out of the reaction vessel to stop the reaction.

(4). The fluorescent intensity of the label in the wells of the microplate is measured with a

fluorometer and is reciprocally proportional to the amount of the inhibitor.

This assay is a specific example of the general assay given in section 2 of the "Detailed Description of the Invention".

Example 3

Telomerase and its inhibitor assay using the pin method

Materials: Tris-acetate buffer (pH 8.5), potassium acetate, β-mercaptoethanol, spermidine, $MgCl_2$, EDTA, streptavidin are from Sigma. dATP, dTTP, fluo-dGTP, telomerase S10, RNase, biotin-oligonucleotide primer may be purchased from Boehringer Mannheim or elsewhere. Inhibitor (7-deaza-dATP).

Mehtod:

1. Preparation of a coated 96-pin lid:

(1). 5 g skim milk powder dissolved in 100 ml PBS (pH 7.2) is added to the wells of a microplate and incubated at 37^0 C for 2 hr. The microplate is washed three times with PBST.

(2). Streptavidin or avidin is dissolved in a buffer to the concentration 5 ug/ml and 100 ul/well is added to each well of the microplate blocked with skim milk. The 96-pin lid is inserted into the wells of the microplate and incubated at 37^0 C for 3 hr, and is then taken out and rinsed with PBST.

2. Telomerase activity assay:

(1). A series of concentration of telomerase in a reaction mixture containing 50 mM Tris-acetate pH 8.5, 50 mM potassium acetate (KAc), 5 mM β-mercaptoethanol, 1mM spermidine, 1mM $MgCl_2$, 0.5-2 mM dATP, 0.5-2 mM dTTP, 1.5 uM fluo-dGTP, 1 uM biotin-oligonucleotide primer $(TTAGG)_3$, are added to wells of a microplate and the mixture is incubated at 30^0 C for 1

hr. The reaction of DNA synthesis is stopped by adding the stop solution (10 mM Tris-HCl, pH7.5, 230 mM EDTA and 100 ug/ml RNase) at 37° C for 15 min.

(2). Isolation of the fluorescent label-DNA with biotin-primer (fluo-DNA-biotin) from the reaction vessel is done by the binding of the fluo-DNA-biotin complex to the streptavidin (avidin)-coated 96 pin lid after the 96-pin lid has been inserted into the reaction vessel.

(3). The fluorescent intensity of fluo-dGTP remaining in the reaction vessel is measured with a fluorometer after the 96-pin lid has been taken out of the reaction vessel and is reciprocally proportional to the activity of telomerase.

3. Telomerase inhibitor assay:

(1). Varying amounts of the inhibitor in the reaction mixture (50 ul/well) are added to the wells of a microplate.

(2). A fixed activity of telomerase in the reaction mixture (50 ul/well) is added to the wells containing an inhibitor and the controls. Incubate the wells of the plate at 30° C for 1-2 hr and stop the DNA synthesis with the stop solution.

(3). Isolation of the fluorescent label-DNA with biotin-primer (fluo-DNA-biotin) from the reaction vessel is done via the binding of fluo-DNA-biotin complex to the streptavidin (avidin)-coated 96 pin lid after the 96-pin lid has been inserted into the reaction vessel.

(4). The fluorescent intensity of fluo-dGTP remaining in the reaction vessel is measured with a fluorometer after the 96-pin lid has been taken out of the reaction vessel and is directly proportional to the amount of the inhibitor.

This assay is a specific example of the general assay given in section 3 of the "Detailed Description of the Invention".

Example 4

Competitive assay of E. coli K88 fimbriae, receptor and inhibitor using the pin method

Materials: Na_2HPO_4, NaH_2PO_4, NaCl, dimethyl sulfoxide (DMSO) are from Sigma. NHS-fluorescein is from Molecular Probes. A mucus receptor from a piglet and E. coli K88 fimbriae are prepared in a laboratory. Inhibitors are from different sources.

Method:

1. Preparation of fluo-fimbriae: 5 mg NHS-fluorescein in 100 ul DMSO is mixed with 10 mg fimbriae in 1 ml PBS (pH 7.2) followed by incubation at room temperature for 3 hr.

2. Preparation of a coated 96-pin lid

(1). 5 g skim milk powder dissolved in 100 ml PBS (pH 7.2) is added to the wells of a microplate and incubated at 37° C for 2 hr. The microplate is washed three times with PBST.

(2). Receptor is dissolved in PBS (pH 7.2) to a concentration of 5 ug/ml and 100 ul/well is added to each well of the microplate blocked with skim milk. The 96-pin lid is inserted into the wells of the microplate and incubated at 37° C for 3 hr, and is then taken out and rinsed with PBST.

3. Receptor assay:

(1). A series of concentrations of the receptor in a buffer (50 ul/well) are added to the wells of a microplate.

(2). A fixed amount of the fluo-fimbriae (50 ul/well) is added to each well containing the receptor and the control.

(3). Competitive binding reactions between the immobilized receptor and the free receptor (competitor) to the fluo-fimbriae are initiated by inserting the 96-pin lid coated with the

receptor into the wells. Incubate at 37^0 C for 1 hr . Then the 96-pin lid is taken out of the vessel to stop the reaction.

(4). The fluorescent intensity of the fluo-fimbriae remaining in the wells of the microplate is determined using a fluorometer and is directly proportional to the amount of the receptor (competitor).

4. Fimbriae or E. coli numeration assay:

(1). A series of concentration of the fimbriae or E. coli cell in a buffer (50 ul/well) are added to the wells of a microplate.

(2). A fixed amount of the fluo-fimbriae (50 ul/well) is added to each well containing the fimbriae or E. coli and control wells.

(3). Competitive binding reactions between the fimbriae or E. coli (competitor) and the fluo-fimbriae to the immobilized receptor are initiated by inserting the 96-pin lid coated with the receptor into the wells. Incubate at 37^0 C for 1 hr. Then the 96-pin lid is taken out of the vessel to stop the reaction.

(4). The fluorescent intensity of the fluo-fimbriae remaining in the wells of the microplate is determined using a fluorometer and is directly proportional to the amount of the fimbriae or E. coli cells (competitor).

5. Inhibitor of the fimbriae-receptor binding assay:

(1). A series of concentration of the inhibitor in a buffer (50 ul/well) are added to the wells of a microplate.

(2). A fixed amount of the fluo-fimbriae (50 ul/well) is added to each well containing the inhibitor and the controls.

(3). The reactions between the inhibitor and the immobilized receptor for binding to fluo-fimbriae or between the inhibitor and the fluo-fimbriae for binding to the immobilized receptor are initiated by inserting the 96-pin lid coated with the receptor into the wells. After incubation at 37^0 C for 1 hr the 96-pin lid is taken out of the vessel to stop the reaction.

(4). The fluorescent intensity of the fluo-fimbriae remaining in the wells of the microplate is determined using a fluorometer and is directly proportional to the amount of the inhibitor (competitor).

This assay is a specific example of the general assay given in section 4 of the "Detailed Description of the Invention".

(3). The reactions between the inhibitor and the immobilized receptor for binding to fluo-fimbriae or between the inhibitor and the fluo-fimbriae for binding to the immobilized receptor are initiated by inserting the 96-pin lid coated with the receptor into the wells. After incubation at 37^0 C for 1 hr the 96-pin lid is taken out of the vessel to stop the reaction.

(4). The fluorescent intensity of the fluo-fimbriae remaining in the wells of the microplate is determined using a fluorometer and is directly proportional to the amount of the inhibitor (competitor).

This assay is a specific example of the general assay given in section 4 of the "Detailed Description of the Invention".

CLAIMS

We claim:

1. A method using the surface of a pin-like device for determining the biological activity of bioactive substance based on the transfer of a moiety between reactants comprising:

a. the surface of a pin-like device coated with reactant 1.

b. insertion of said surface of a pin-like device into a reaction vessel containing reactant 2 which has bioactivity and reactant 3 linked with a label; the labeled moiety of reactant 3 being transferred to said reactant 1 due to the biological activity of said reactant 2. Then said surface of a pin-like device being taken out of the reaction vessel to stop the reaction.

c. measuring the amount of said reactant 3 linked with a label remaining in the reaction vessel, wherein the amount of said reactant 3 linked with a label in said reaction vessel is reciprocally proportional to the activity of said reactant 2.

2. The method of claim 1 wherein said surface of a pin-like device is any shape such as a cylinder, spire, star, cuboid, cone.

3. The method of claim 1 wherein said reactant 1 is an acceptor of the moiety of said reactant 3 which is a substrate for said reactant 2 which is an enzyme.

4. The method of claim 1 wherein said label is selected from radioactive tags, or fluorescent, luminescent or chromogenic molecules.

5. The method of claim 3 wherein said enzyme is an enzyme capable of transferring a moiety from one molecule to another.

6. The method of claim 3 wherein said acceptor is selected from casein, or other protein or a peptide.

7. The method of claim 3 wherein said substrate is ^{32} P-ATP.

8. The method of claim 5 wherein said enzyme is a protein kinase.

9. The method for determining the amount of an inhibitor of a biological activity of said reactant 2 comprising:

a. the surface of a pin-like device coated with reactant 1.

b. insertion of said surface of a pin-like device into a reaction vessel containing a known amount of reactant 2, reactant 3 linked with a label and an unknown amount of reactant 4 being an inhibitor of reactant 2. The reactions among reactant 1, reactant 2 and reactant 3 being interfered by reactant 4 due to the inhibition of the biological activity of reactant 2. Then said surface of a pin-like device being taken out of the reaction vessel to stop the reaction.

c. measuring the amount of said reactant 3 linked with a label remaining in the reaction vessel, wherein the direct relationship exists between reactant 3 remaining in the reaction vessel

and reactant 4.

10. A method using the surface of a pin-like device for detecting the biological activity of a bioactive substance utilizing the degradation of a substrate comprising:

a. the surface of a pin-like device coated with reactant 1 linked with a label.

b. insertion of said surface of a pin-like device into a reaction vessel containing reactant 2 which has biological activity, said reactant 1 being hydrolyzed due to the activity of said reactant 2 to release the label into said reaction vessel. Said surface of a pin-like device being taken out of the reaction vessel to stop the reaction.

c. measuring the amount of the label in the reaction vessel, wherein the amount of the label in the vessel is directly proportional to the biological activity of said reactant 2.

11. The method of claim 10 wherein said reactant 2 is an enzyme and said reactant 1 is a substrate for the enzyme.

12. The method of claim 11 wherein said substrate is a polymeric or an oligomeric substrate.

13. The method of claim 10 wherein said enzyme is an enzyme that is able to cleave the substrate.

14. The method of claim 12 wherein said polymeric substrate is selected from a carbohydrate, DNA, RNA, protein, PEG, or polypeptide.

15. The method of claim 12 wherein said oligomeric substrate is selected from a peptide, oligosaccharide, or oligonucleotide.

16. The method of claim 11 wherein said enzyme is a protease or proteinase and said substrate is a protein.

17. The method of claim 11 wherein said enzyme is a carbohydrate hydrolase and said substrate

is a carbohydrate.

18. The method of claim 11 wherein said enzyme is a DNase and said substrate is a DNA.

19. The method of claim 11 wherein said enzyme is a RNase and said substrate is a RNA.

20. The method of claim 11 wherein said enzyme is a peptidase and said substrate is a peptide.

21. The method of claim 11 wherein said enzyme is a oligosaccharide hydrolase and said substrate is a oligosaccharide.

22. The method of claim 10 wherein said label is selected from radioactive tags, fluorescent molecules, luminescent molecules and chromogenic dyes.

23. A method using the surface of a pin-like device for detecting the amount of an inhibitor to a bioactive substance comprising:

a. the surface of a pin-like device coated with reactant 1 linked with a label.

b. insertion of said surface of a pin-like device in to a reaction vessel containing a known amount of reactant 2 which has biological activity, an unknown amount of reactant 3 being an inhibitor of reactant 2. The cleavage of reactant 1 by reactant 2 being inhibited due to the activity of reactant 3. Said surface of a pin-like device being taken out of the reaction vessel to end the reaction.

c. measuring the amount of the label in the reaction vessel, wherein the amount of the label in the vessel is reciprocally proportional to the amount of reactant 3 (inhibitor).

24. A method using the surface of a pin-like device for detecting a biological activity of a bioactive substance comprising:

a. the surface of a pin-like device coated with reactant 1.

b. insertion of said surface of a pin-like device into a reaction vessel containing product 1

formed from reactants 3 linked with either a label or a binding agent due to the activity of reactant 2. Binding to product 1 by reactant 1 coated on the surface of a pin-like device caused by biological activity. Said surface of a pin-like device being taken out of the reaction vessel to stop the binding reaction between reactant 1 and product 1.

c. measuring the amount of labeled reactant 3 remaining in the reaction vessel wherein the amount of the label in the vessel is reciprocally proportional to the activity of reactant 2.

25. The method of claim 24 wherein said reactant 1 is a binding ligand to said product 1.

26. The method of claim 24 wherein said reactant 2 is an enzyme and said reactant 3 is a labeled substrate for the enzyme.

27. The method of claim 24 wherein said label is selected from radioactive tags, or fluorescent, luminescent or chromogenic molecules.

28. The method of claim 25 wherein said binding ligand is avidin or streptavidin.

29. The method of claim 26 wherein said enzyme is telomerase.

30. The method of claim 26 wherein said substrates are a fluo-dGTP or a fluo-dNTP and a biotin labeled primer.

31. The method of claim 24 wherein said product 1 is DNA labeled with biotin and a fluorescent molecule.

32. A method using the surface of a pin-like device for detecting the amount of an inhibitor of the bioactive substance comprising:

a. the surface of a pin-like device coated with reactant 1.

b. insertion of said surface of a pin-like device into a reaction vessel containing a known amount of reactant 2, reactant 3, an unknown amount of reactant 4 being an inhibitor of reactant

2 and product 1. The formation of product 1 from reactant 3 due to the activity of reactant 2 being inhibited by reactant 4. Binding to the product 1 by reactant 1 coated on the surface of a pin-like device caused by a biological activity. Said surface of a pin-like device being taken out of the reaction vessel to end the binding reactions.

c. determining the amount of labeled reactant 3 remaining in the vessel wherein the amount of the label in the vessel is directly proportional to the amount of the inhibitor.

33. A competitive method using the surface of a pin-like device for measuring the amount of a bioactive substance utilizing a competitive binding between the bioactive substances comprising:

a. the surface of a pin-like device coated with reactant 1.

b. insertion of said surface of a pin-like device into a reaction vessel containing a known amount of reactant 3 linked with a label and an unknown amount of reactant 2. The competitive reactions existing between reactant 2 and reactant 3 to bind to reactant 1 or between reactant 1 and reactant2 to bind to reactant 3. Said surface of a pin-like device being taken out of the reaction vessel to stop the competitive reactions.

c. determining the amount of labeled reactant 3 remaining in the reaction vessel wherein the amount of the labels in the vessel is directly proportional to the amount of reactant 2.

34. The method of claim 33 wherein said reactant 1 is a receptor, said reactant 2 is a receptor binding ligand and said reactant 3 is the labeled form of reactant 2.

35. The method of claim 33 wherein said reactant 1 is a receptor binding ligand, reactant 2 is a receptor and said reactant 3 is the labeled form of reactant 2.

36. The method of claim 33 wherein said reactant 1 is a lectin, said reactant 2 is lectin binding ligand and said reactant 3 is the labeled form of reactant 2.

37. The method of claim 33 wherein said reactant 1 is a lectin binding ligand, said reactant 2 is a lectin and said reactant 3 is the labeled form of reactant 2.

38. The method of claim 33 wherein said reactant 1 is an enzyme, said reactant 2 is an inhibitor and said reactant 3 is the labeled form of reactant 2.

39. The method of claim 33 wherein said reactant 1 is an inhibitor, said reactant 2 is an enzyme and said reactant 3 is the labeled form of reactant 2.

40. The method of claim 33 wherein said label is selected from radioactive tags, or fluorescent, luminescent, or chromogenic molecules.

41. The method of claim 34 wherein said receptor is a fimbriae receptor, said receptor binding ligand is a fimbriae and said receptor binding ligand linked with a label is a fimbriae linked with a label.

42. The method of claim 33 wherein said reactant 1 is a receptor, said reactant 2 is an inhibitor of the binding between the receptor and a receptor binding ligand linked with a label (said reactant 3).

43. The method of claim 33 wherein said reactant 1 is a lectin, said reactant 2 is an inhibitor of the binding between the lectin and a lectin binding ligand linked with a label (said reactant 3).

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